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Complement-dependent lysis of tumor cells by a baboon IgM antibody to a tumor-associated antigen

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Summary. We developed a high-titer polyclonal antiserum to a glycoprotein tumor-associated antigen (TAA) by immunization of a baboon with the purified glycoprotein antigen. The baboon serum was fractionated into IgG and IgM components by DEAE Affi-Gel blue chromatography. The ability of the baboon IgM anti-TAA antibody to effect tumor cell lysis in the presence of complement was tested using a chromium-release assay. The baboon antibody was able to lyse melanoma target cells (20.8%–71.4% cytolysis), breast carcinoma cells (36.5%–38.9% cytolysis), and a neuroblastoma cell line (35.5% cytolysis) in the presence of complement but did not effect significant lysis of autologous lymphoblastoid cell lines (4.9% cytolysis) or peripheral blood lymphocytes from healthy volunteers (12.6% cytolysis). Cytolysis of melanoma target cells was completely inhibited by preabsorption of the IgM anti-TAA antibody with UCLA-SO-M14 (M14) cells and partially inhibited by preabsorption with several other melanoma cell lines. There was no significant inhibition of tumor cell lysis after preabsorption of the antibody with lymphoblastoid cell lines. Complement-dependent lysis of M14 targets could be blocked by addition of the purified antigen to the antibody prior to incubation with the tumor cells. Our results suggest that the glycoprotein TAA resides on the tumor cell surface and that the baboon IgM anti-TAA antibody recognizes the antigen on the cell surface and is able to fix complement and effect the lysis of the tumor cells.

Key words: Tumor-associated antigen – Complement-dependent cytotoxicity – Polyclonal antibodies – Monoclonal antibodies – Glycoprotein antigen

Introduction

Tumor progression involves a series of complex interactions between the host and tumor cell populations. Certain neoantigens, so called tumor-associated antigens (TAA), that reside on the surface of tumor cells, can be recognized by the host as foreign targets for immunological destruction. Although several TAAs have been described, not all have been shown to be immunogenic in man. It is the immunogenic TAA that would be the logical targets for immunotherapy in addition to immunodiagnosis of human cancer.

Monoclonal and polyclonal antibodies have been developed to a number of TAAs. These antibodies have allowed detailed in vitro studies of cell-surface antigens on human cancers and have increased our understanding of immune responses to cancer. A glycoprotein antigenic complex was first detected by reacting autologous serum with urine from melanoma patients [20]. This antigenic complex exhibited a molecular mass of 590–620 kDa by Sephacryl S-300 gel filtration chromatography and sedimented in the region of 28%–29% sucrose by density gradient ultracentrifugation [10]. Both monoclonal and polyclonal antibodies have been developed to this glycoprotein TAA in our laboratory [8, 10]. We have also demonstrated the augmentation of anti-TAA antibodies of the IgM and IgG classes in stage II and III melanoma patients following the administration of a whole melanoma cell vaccine [9]. Unfortunately these autologous and allogeneic antibodies from cancer patients are available in only limited quantities and do not allow detailed study of interactions between TAA and corresponding antibodies. Because of phylogenetic proximity in the immune systems of humans and primates, we used a baboon as the source of polyclonal antiserum to this glycoprotein TAA. The xenoantiserum was produced by immunization with a purified antigen, which allowed for the development of a more specific antibody than those obtained by immunization with whole tumor cells [7].

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In this report we describe the production and purification of a baboon IgM anti-TAA antibody and its ability to effect tumor cell lysis in the presence of complement.

Materials and methods

Preparation of TAA. TAA was purified as described elsewhere [10]. Briefly, a 24-h urine sample collected from a melanoma patient was centrifuged at 2600 *g* for 10 min and filtered through a Whatman no. 1 filter-paper (Whatman International, Maidstone, England) to remove all sediment. The clarified urine was concentrated 100-fold using an Amicon hollow-fiber concentrator equipped with an H1 P10-8 cartridge (Amicon, Danvers, Mass.). Concentrated material was chromatographed on a Sephacryl S-200 column (Pharmacia LKB, Piscataway, N. J.) using 0.025 M phosphate-buffered saline supplemented with 0.02% sodium azide as eluent at a flow rate of 25 ml/h. Fractions under each peak observed by absorbance at 280 nm were pooled separately, concentrated and tested for antigen activity using a double-determinant enzyme-linked immunosorbent assay (ELISA) as described previously [8]. We utilized murine monoclonal IgM antibody (AD1-40F4) and allogeneic IgM and IgG antibodies. The antigenic activity was invariably found in the first peak.

Immunization protocol. A 12-year-old, 28-kg, male baboon received intramuscular or intravenous injections of the tumor-associated antigen (TAA) at 4-week intervals. TAA (100 µg protein) in 0.5 ml phosphate-buffered saline was mixed with an equal volume of Mylanta II (Stuart Pharmaceuticals, Wilmington, Del.) and administered intramuscularly to the baboon at monthly intervals. After six intramuscular injections, the baboon was bled at monthly intervals and serum anti-TAA levels were measured using ELISA as described below. The baboon was boosted by intravenous injection with 20 µg TAA protein 3–5 days before each bleed. Approximately, 75 ml blood was collected per bleed with a yield of about 30–35 ml serum.

Isolation of IgM antibody. The IgM anti-TAA antibody was enriched from the baboon serum by DEAE Affi-Gel blue chromatography (Bio-Rad, Richmond, Calif.). A 15-ml aliquot of the baboon serum was dialyzed for 24 h in 0.02 M K_2HPO_4 supplemented with 0.02% NaN_3 at pH 8.0 with three changes of the buffer. The dialyzed serum was applied to a 50-ml bed-volume of the gel equilibrated with 0.02 M potassium phosphate buffer (pH 8.0) supplemented with 0.02% NaN_3. After flushing the column with 150 ml phosphate buffer (pH 8.0) the IgM fraction was eluted with the phosphate buffer supplemented with 0.5 M NaCl (pH 8.0). The IgM fraction was concentrated to 15 ml (3.6 mg protein/ml) using a PM10 ultrafiltration membrane (Amicon) and dialyzed at 4°C against Roswell Park Memorial Institute 1640 (RPMI) medium (J. R. Scientific, Woodland, Calif.) for 24 h with three to four changes of the medium (1000 ml each) to remove NaN_3 before use in the cytotoxicity assay.

ELISA. Purified TAA was adsorbed to polystyrene wells of ELISA plates (Dynatech Laboratories Inc., Chantilly, Va.) at a concentration of 120 ng protein/well in carbonate buffer, pH 9.6, by incubation at 37°C for 3 h. After washing, using a Titertek microplate washer (Flow Laboratories, McLean, Va.), 100 µl baboon IgM anti-TAA antibody, diluted serially in 0.025 M phosphate-buffered saline and Tween-20, pH 7.2 (PBS/Tween), was added to the wells containing TAA and to the wells without the antigen. After incubation at 37°C for 45 min and subsequent washing, 100 µl alkaline-phosphatase-conjugated anti-(human IgM)antibody (Sigma Chemical Co., St. Louis, Mo.) at 1:500 dilution was added to each well. After further incubation at 37°C for 45 min and washing, 200 µl *p*-nitrophenyl phosphate (Sigma) at 1 mg/ml in diethanolamine buffer, pH 9.8, was added to all wells. The plates were incubated at room temperature for 3 h and the color development was assessed at 405 nm using a Titertek Multiskan plate reader (Flow Laboratories). Each washing step consisted of five washes with PBS/Tween. The antibody titer was defined as the highest dilution that resulted in an absorbance of 0.05.

Cell lines. Tumor cell lines (melanoma, breast, and neuroblastoma) and lymphoblastoid cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (Flow Laboratories), streptomycin/penicillin/fungizone antibiotics, and 0.01 M HEPES buffer, and incubated at 37°C in a humidified incubator until the cell growth reached confluency.

Preparation of target cells. Tumor cells growing as a monolayer were harvested from flasks at the confluent growth phase using 0.25% trypsin and 0.01 M ethylene diamine tetraacetate (EDTA). The harvested single-cell suspensions were washed three times with 20 ml RPMI medium by centrifugation (500 *g*, 5 min). Peripheral blood lymphocytes were isolated from heparinized blood of healthy volunteers using a Ficoll-Hypaque (Pharmacia) density gradient [1]. After washing the mononuclear cells with RPMI medium, the monocytes were depleted by layering the cell suspension in 10% human AB serum onto a tissue-culture dish and incubating it at 37°C for 1.0 h in a humidified incubator with 5% CO_2 atmosphere. The non-adherent cells were collected, washed and resuspended in RPMI medium. Samples of 2×10^6 target cells were chromated with 250 µCi sodium [^{51}Cr]chromate solution for 1 h at 37°C. Chromium-labelled target cells were washed twice with RPMI medium supplemented with 10% fetal calf serum and once with medium only. Target cells were then resuspended in RPMI medium for use in the complement-dependent cytotoxicity assay.

Absorption of isolated antibodies. Tumor cells and lymphoblastoid cells were counted and resuspended in RPMI medium with the baboon IgM anti-TAA antibody (dilution 1:6) in microcentrifuge tubes (Robbins Scientific, Mountain View, Calif.) at the following densities: 1.0×10^4, 1.0×10^5, 1.0×10^6, and 1.0×10^7 cells/ml. The cell supensions were incubated with the antibody at 4°C for 8–12 h and then centrifuged at 7000 *g* for 5 min. The supernates were aspirated and used as the antibody source in the cytotoxicity assay.

Blocking of anti-TAA activity. The baboon IgM anti-TAA antibody was incubated with protein-G-positive streptococcal cell suspension (Sigma) at 4°C for 12 h to remove any contaminating antibodies of the IgG type. The suspension was then centrifuged at 500 *g* for 10 min and the supernate collected. Serial doubling dilutions of the antibody were made in RPMI medium and mixed with the purified TAA, which was diluted 1:2 in PBS, incubated at 37°C for 1.0 h and used in the cytotoxicity assay to determine reduction in the antibody activity. The RPMI buffer was also absorbed with protein G streptococcal cells and used in the complement-dependent cytotoxicity (CDC) assay as a control to document that inhibition was not due to material leached from the bacterial cell.

Complement-dependent cytotoxicity assay. The cytotoxic effect of the baboon IgM anti-TAA antibody in the presence of complement was measured using a ^{51}Cr-release assay. Target cells were labeled with ^{51}Cr as described above and placed in 96-well microtiter plates (Costar, Cambridge, Mass.) at a concentration of 100 000 cells/well, mixed with 50 µl IgM anti-TAA antibody, diluted in RPMI medium, and incubated for 1.0 h at 4°C. Baby rabbit complement (Cedarlane Laboratories, Ontario, Canada) was then added (final dilution 1:10) and incubated for 2 h at 37°C. The plates were centrifuged at 500 *g* for 5 min and 100 µl supernatant was aspirated from each well to determine released ^{51}Cr. Spontaneous release was determined from wells containing only target cells in RPMI medium and total release was obtained from wells containing target cells lysed by 10% sodium dodecyl sulfate. Cytotoxicity was expressed by the formula:

$$\text{cytolysis (\%)} = \frac{\text{(experimental release} - \text{spontaneous release)}}{\text{(total release} - \text{spontaneous release)}} \times 100$$

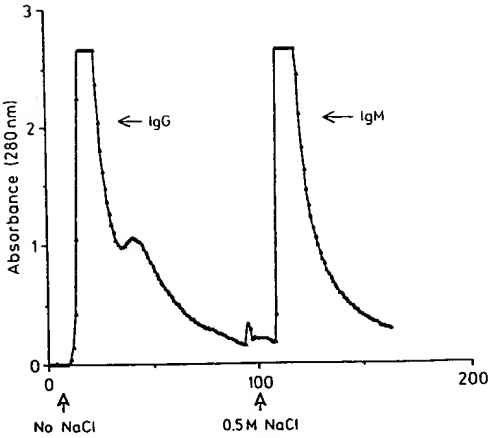

Fig. 1. Elution profile of the baboon serum from a DEAE Affi-Gel blue column. The baboon IgG anti-(tumor-associated antigen)(TAA) antibody is eluted in the first peak using 0.02 M potassium phosphate buffer supplemented with 0.02% NaN_3. The IgM fraction is eluted by adding 0.5 M NaCl to the potassium phosphate buffer. Fractions were read at 280 nm

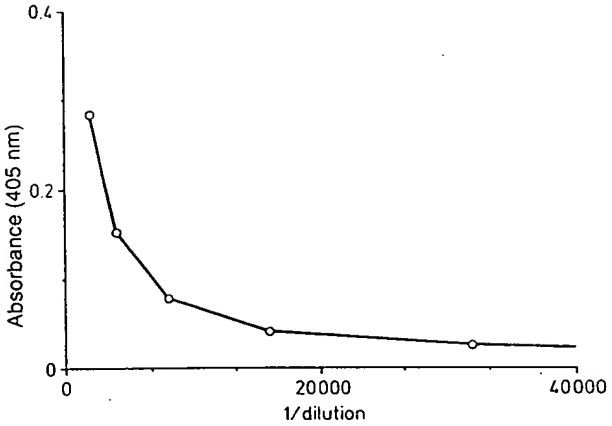

Fig. 2. The baboon IgM anti-TAA antibody titer was determined using ELISA. Purified TAA was adsorbed to wells at a concentration of 120 ng/well. Serial dilutions of the IgM antibody are added to the wells followed by incubation with an alkaline-phosphatase-conjugated anti-(human IgM) antibody. The antibody titer was defined as the dilution that resulted in an absorbance of 0.05 at 405 nm

Results

Antibody purification by DEAE Affi-Gel blue

Figure 1 shows the elution profile of the baboon serum from a DEAE Affi-Gel blue column using a step sodium chloride gradient. Two major and three minor peaks were eluted from the column. The first major peak contained IgG antibody and the second major peak contained IgM. These fractions were concentrated to represent the original volume of the serum that was placed onto the column. As assessed by ELISA, the first peak contained no IgM; however, the second (IgM) peak contained trace amounts of IgG. Protein concentrations were 4.43 mg/ml for the IgG antibody and 3.6 mg/ml for the IgM antibody.

Anti-TAA activity

The whole baboon serum had an IgG anti-TAA titer greater than 1:1 024000 and an IgM anti-TAA titer of 1:64000 as measured by ELISA. The anti-TAA titer in the IgG fraction was 1:250000 and in the IgM fraction it was 1:16000 (Fig. 2). Thus, on a volume basis, further purification of the IgM fraction significantly reduced the anti-TAA antibody activity perhaps because of loss during concentration of this fraction. Since the IgM fraction was not devoid of IgG and it had an IgG anti-TAA titer of 1:14000, this fraction (the IgM antibody) was treated with protein-G-positive streptococcal cells prior to use. This treatment eliminated all of the anti-TAA antibody of the IgG type.

Complement-dependent cytotoxicity (CDC) of the baboon IgM antibody

In preliminary experiments, UCLA-SO-M14 (M14), a human melanoma cell line, was used as the target in the ^{51}Cr-release assay. At an antibody dilution of 1:6

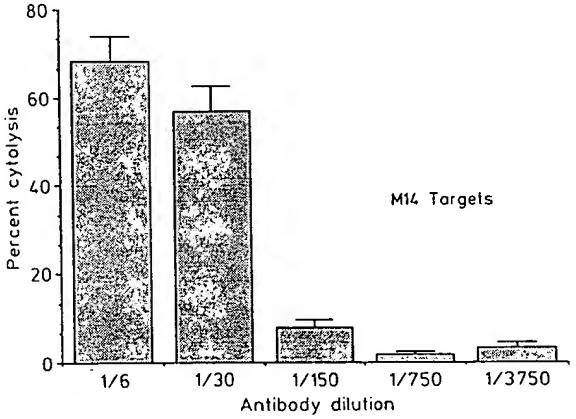

Fig. 3. Tumor cell lysis by the baboon IgM anti-TAA antibody in a complement-dependent cytotoxicity assay. UCLA-SO-M14 cells (M14, melanoma cell line) were used as the target at a concentration of 10000 cells/well. Serial antibody dilutions were added to the cells followed by the addition of baby rabbit complement. Percentage cytolysis was determined by the amount of chromium released into the medium.

(18.5 mg/dl IgM) the percentage cytolysis of M14 was 68.3%. This cytolysis decreased with further dilution of the antibody (Fig. 3). Use of other melanoma cell lines, UCLA-SO-M7 (M7), UCLA-SO-M10 (M10), UCLA-SO-M12 (M12), UCLA-SO-M15 (M15), UCLA-SO-M16 (M16), UCLA-SO-M101 (M101), and UCLA-SO-M109 (M109), as targets in subsequent experiments revealed that the cytolysis ranged from 20.8% to 71.4% at an antibody dilution of 1:6 for the different cell lines (Table 1). The degree of cytolysis was dependent on the antibody dose (data not shown).

Other target cell lines used were two breast carcinoma (CPR and MCF) cell lines, five neuroblastoma (CHP, Lan-1, Lan-2, Lan-5 and SHF) cell lines, peripheral blood lymphocytes (PBL) from healthy volunteers, and five lymphoblastoid [UCLA-SO-L15 (L15), UCLA-SO-L14 (L14), UCLA-SO-L10 (L10), UCLA-SO-L21 (L21), and

Table 1. Complement-dependent cytotoxicity against various cell lines

Cell line	Cytolysis[a] ± SD (%)
Melanoma	
M7	43.4±2.2
M10	40.0±5.0
M12	71.4±0.4
M14	68.3±5.6
M15	36.5±2.8
M16	20.8±4.0
M101	25.3±4.6
M109	29.0±2.7
Breast carcinoma	
CPR	36.5±9.1
MCF	38.9±13.4
Neuroblastoma	
CHP	35.5±1.5
Lan-1	39.3±3.7
Lan-2	34.5±7.6
Lan-5	31.6±11.6
SHF	29.2±8.3
Lymphoblastoid	
L15	4.9±4.9
L14	9.7±4.1
L10	5.8±3.6
L21	7.9±5.3
L24	5.2±4.4
PBL[b] of healthy controls	12.6±3.21

[a] Percentage lysis was computed by the formula given in the text. In this set of experiments, spontaneous release of radioactivity was 10% – 15%
[b] Peripheral blood lymphocytes

UCLA-SO-L24 (L24)] cell lines. As noted in Table 1, complement-dependent cytotoxicity against the breast carcinoma cell lines was 36.5% (CPR) and 38.9% (MCF). For neuroblastoma targets it ranged from 29.2% to 39.3%. On the other hand, the range of cytolysis for lymphoblastoid cells was from 4.9% to 9.7%. Under the same experimental conditions the cytolysis of and PBL from healthy volunteers was 12.6%.

Effect on CDC of absorption of the baboon IgM antibody with various cell lines

Since the baboon IgM anti-TAA antibody was able to lyse tumor targets, in the presence of complement, but not normal or lymphoblastoid cells, it was logical to assume that the target antigen (TAA) was located on the cell surface. To confirm this assumption, the antibody was reacted with 1.0×10^3 to 1.0×10^7 tumor cells and 1.0×10^3 to 1.0×10^7 cells from the paired autologous lymphoblastoid, UCLA-SO-L14 (L14), cell line. The absorbed antibody was used at a final dilution of 1:45 in the CDC assay against the M14 target. The cytolysis of the M14 target was completely inhibited by absorption of the antibody with 1.0×10^7 M14 cells. The cytotoxicity to the M14 cells after absorption of the antibody with L14 cells remained virtually unchanged (Fig. 4). To determine whether this inhibition of CDC of M14 cells could be observed by absorption of the antibody with other cell lines, similar experiments were performed where ten different melanoma cell lines and an erythroleukemia cell line, K562, were used to pretreat the baboon IgM antibody. The antibody was absorbed separately with 1.0×10^4 to 1.0×10^7 cells of each of the cell lines at 4° C for 16 h and tested in the chromium-release assay with M14 cells used as the target. The results were expressed as the percentage inhibition of CDC of 1.0×10^4 M14 cells. The melanoma cell lines which showed significant inhibition were M7 (26.4%), M10 (23.5%), M12 (33.5%), M14 (96.3%), and M24 (43.2%). The L14 and K562 cell lines exhibited less than 10% inhibition of CDC (Fig. 5).

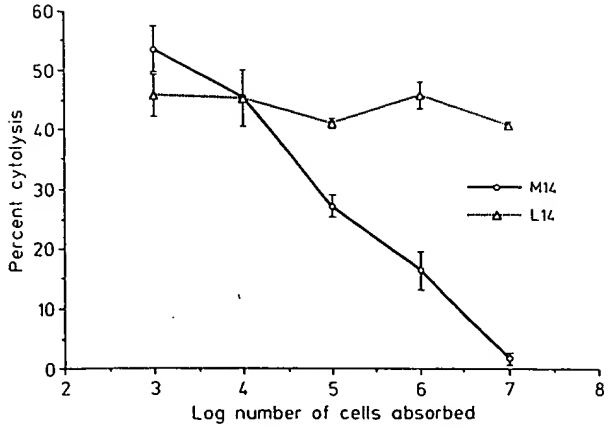

Fig. 4. Specificity of inhibition of cytolysis of the M14 target cells by preabsorption of the baboon IgM anti-TAA antibody with M14 and L14 cells. After absorption with 1.0×10^7 M14 cells there was complete inhibition of the M14 target cell lysis. However, there was no significant inhibition of M14 cell lysis by preabsorption of the baboon antibody with L14 cells (lymphoblastoid cell line autologous to M14)

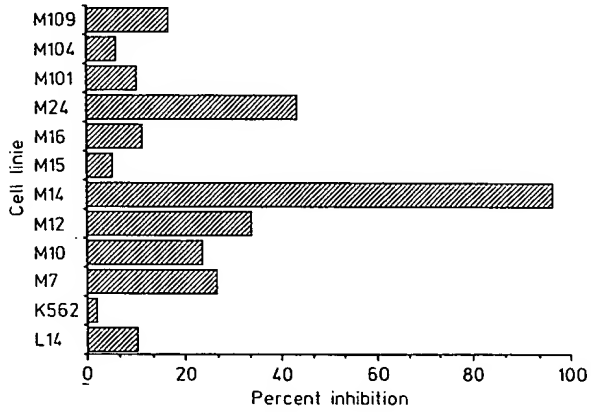

Fig. 5. Inhibition of cytolysis of M14 cells, mediated by baboon IgM anti-TAA antibody, by preabsorption of the antibody with various melanoma cell lines. M7, M10, M12, M14, M24, and M109 led to significant inhibition of lysis of the M14 target cells. Absorption with melanoma cell lines M15, M16, M101 and M104 did not lead to significant inhibition. Similarly, the erythroleukemia line K562 did not significantly alter tumor cell lysis.

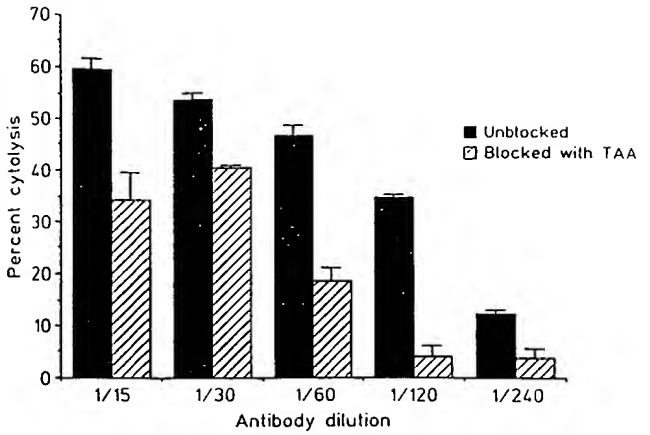

Fig. 6. Inhibition of baboon antibody-mediated cytolysis of M14 cells by purified TAA. Addition of purified TAA to the baboon antibody at 1 : 120 dilution reduced the cytolysis from 34.8% to 4.2%

Blocking of IgM anti-TAA CDC to M14 cells with purified TAA

In order to confirm that the complement-dependent cytotoxicity of the polyclonal baboon IgM anti-TAA antibody was indeed due to the interaction of the antibody with the antigen on the cell surface, the antibody was pretreated with the purified antigen prior to use in the chromium-release assay. For this purpose, purified TAA was added to the serial antibody dilutions as a blocker and allowed to incubate at 37°C for 45 min. The blocked antibody mixtures were then used in the chromium-release assay. The cytotoxic effect of the baboon IgM antibody to M14 cells was significantly reduced by pretreatment with the purified TAA (Fig. 6). This reduction was most pronounced (from 34.8% to 4.2% cytolysis) when the antibody was at a dilution of 1 : 120. Cytotoxicity of the protein G plus complement to the M14 cells was only 7.5%. The TAA antigen plus complement exhibited less than 1% cytolysis of the M14 cells.

Discussion

The specificity of surface antigens of human cancer cells has been the subject of debate for many years [18]. Xenoantisera to membrane-associated antigens of malignant melanoma have been developed in many animal models including rabbits [5, 16, 22], guinea pigs [11], monkeys [6, 3, 15, 17], and chimpanzees [21]. In many of these studies, the investigators were not able to demonstrate specificity of the antibodies to melanoma-associated antigens and this was often a criticism of using xenogeneic antisera. The antibodies in most of these early studies were generated by using whole melanoma cell preparations as immunogens, which might have elicited nonspecific antibodies reactive with other antigens present on the surface of tumor cells. In our study, we have been able to obtain high-titer antibodies to a glycoprotein TAA by immunizing the baboon with purified TAA prepared from the urine of melanoma patients.

We have previously reported that the glycoprotein TAA is detected in the urine of 64% of melanoma patients but only 5% of normal individuals [10]. While some melanomas do not express the TAA, detection in the urine may also be subject to differences in the turnover and release of the antigen from the cell surface or to interference by complexes resulting from release of the antigen with other membrane fragments [5]. The baboon IgM anti-TAA antibody that we have developed is cytotoxic to melanoma and other tumor cell lines in the presence of complement. The IgM antibody separated from the baboon serum was found to be contaminated with IgG antibody as demonstrated by ELISA; however, the IgG fraction was devoid of IgM antibody. Indeed, the contaminating IgG antibody may interfere with the ability of the IgM antibody to effect cytolysis. In our initial experiments, the IgM anti-TAA antibody was only able to effect lysis of 40%–50% of tumor cells. We felt this was due to competition between the IgG and IgM antibodies for interaction with TAA on the tumor cell surface. This competition was reduced by pretreating the IgM fraction with protein G, which absorbs the contaminating IgG antibody. The IgM antibody was then able effectively to lyse 70%–80% of the cells. The IgG fraction of the baboon serum was not cytotoxic to any of the tumor cell lines tested even in the presence of complement. Lando et al., described a similar occurrence when they studied the cytotoxic antibodies found in the serum of hepatoma-D23-bearing rats [14]. While the IgG fractions were found to have immune complexes formed by interaction between IgG antibodies and a tumor-associated antigen, these were not involved in the cytotoxic activity to D23 hepatoma cells.

We found that the baboon IgM anti-TAA antibody was cytotoxic to some tumor cell lines, including melanoma, breast carcinoma, and neuroblastoma lines, in a complement-dependent cytotoxicity assay. The cytolysis ranged from 20.8% to 71.4% in different cell lines. There was no significant cytolysis; however, to lymphoblastoid cell lines or fresh human peripheral blood lymphocytes. The variability in complement-dependent cell lysis by the baboon antibody to these different cell lines may be related to the amount of TAA expressed on the cell surface or to differences in susceptibility to lysis of the individual cell lines.

The fact that the baboon IgM antibody is able to lyse tumor cells other than melanoma is not unexpected since we have recently been able to detect TAA in the urine and serum of patients with carcinoma of the lung, breast, and colon and patients with sarcomas but only in about 5% of normal volunteers [2, 13].

Cultured cell lines have been demonstrated to have changes in the expression of cell-surface antigens when compared with the parent cell line or tissue of origin [4, 12]. These changes are probably dependent on multiple factors including period of time in culture and interaction of the cells with proteins in the tissue-culture medium. Since the baboon antibody is able to lyse M14 cells but not autologous lymphoblastoid cells (L14), this suggests that the baboon antibody is recognizing a tumor-associated antigen and not simply a nonspecific epitope located on the cell surface or histocompatibility locus antigen (HLA) determinant. Further supporting evidence for a tumor-as-

sociated antigen is the inability of the baboon antibody to lyse human lymphocytes from normal volunteers.

To test further our hypothesis that the baboon antibody was interacting with TAA on the cell surface, we performed absorption studies of the IgM antibody with various cell lines. The antibody was ineffective at lysing tumor target cells after preabsorption with M14 cells but retained essentially all of its activity after absorption with the same number of lymphoblastoid (L14) cells. Other melanoma cell lines used for absorption showed varying degrees of inhibition of CDC. Again, this may be related to the density of antigen on the cell surface. Nores et al. have recently shown that the reactivity of the mouse monoclonal antibody M2590, directed at GM3 ganglioside, depends on the density of GM3 exposed at the cell surface [19]. Although TAA is a glycoprotein antigen, the antigen/antibody interactions on the cell surface may be dependent on the density of the antigen and related confirmational changes of the molecule in the cell membrane.

The ability of viable tumor cells to inhibit CDC by preabsorption of the baboon anti-TAA antibody supports the concept that it is a cell-surface molecule recognized by the antibody. We were further able to inhibit cell lysis by adding purified TAA to the antibody prior to its use in the cytotoxicity assay. At an antibody dilution of 1 : 120, cytotoxicity was reduced from 34.8% to 4.2% (88% inhibition of cytolysis). At higher concentrations of antibody, inhibition by the same concentration of purified TAA was only 35% to 50%. This suggests that at antibody concentrations represented by less than 1 : 120 dilution of the IgM fraction, the concentration of purified TAA was not high enough to neutralize all of the antibody molecules. These blocking experiments are contributing evidence that the antigen recognized by the baboon IgM antibody on the surface of the tumor is the glycoprotein tumor-associated antigen that was used as the immunogen to raise the antibody.

Since the TAA is shed in the urine of cancer patients, this makes it a useful marker for immunodiagnosis and possibly for immunoprognosis. The results of investigations described here suggest that TAA is a cell-surface antigen that is associated with tumor cells and specifically recognized by the baboon anti-TAA antibody. This makes the glycoprotein TAA antigen a potential target for immunotherapy and immunodetection. The baboon anti-TAA antibody (IgM) is able to lyse tumor cells in the presence of complement. This cytolysis can be blocked by the presence of purified antigen. The baboon antibody may prove to be a useful adjunct to monoclonal antibodies in the study of tumor-associated antigens in vitro. It may also be useful as a radiolabeled antibody for in vivo studies using animal models or for radioimmunodetection of tumor in humans.

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References

1. Bain B, Pshyk K (1972) Enhanced reactivity in mixed leucocyte cultures after separation of mononuclear cells on Ficoll-Hypaque. Transplant Proc 4: 163
2. Bauer RL, Gupta RK, Kopald KH, Morton DL (1990) An improved immunoassay to detect antigen specific immune complexes in sera of cancer patients. Proc Am Assoc Cancer Res 31: 263
3. Bruggen J, Sorg C, Macher E (1978) Membrane associated antigens of human malignant melanoma: V. Serological typing of cell lines using antisera from nonhuman primates. Cancer Immunol Immunother 5: 53
4. Bruggen CS, Seibert E, Macher E (1978) Membrane-associated antigens of human malignant melanoma: IV. Changes in expression of antigens on cultured melanoma cells. Cancer Immunol Immunother 3: 259
5. Bystryn J-C (1977) Release of cell-surface tumor-associated antigens by viable melanoma cells from humans. J Natl Cancer Inst 59: 325
6. Carrel S, Dent PB, Liao S-K (1980) Demonstration of the specificity of a monkey antiserum against human melanoma. Cancer Immunol Immunother 8: 197
7. Dent PB, Liao S-K (1982) Heterogeneity of human melanoma-associated antigens revealed by alloantisera and xenoantisera. In: Melanoma antigens and antibodies. Plenum, New York, p 101
8. Euhus DM, Gupta RK, Morton DL (1989) Detection of a tumor-associated glycoprotein antigen in serum and urine of melanoma patients by murine monoclonal antibody (AD1-40F4) in enzyme immunoassay. J Clin Lab Anal 3: 184
9. Euhus DM, Gupta RK, Morton DL (1989) Induction of antibodies to a tumor-associated antigen by immunization with a whole melanoma cell vaccine. Cancer Immunol Immunother 29: 247
10. Euhus DM, Gupta RK, Morton DL (1990) Characterization of a 90–100 kDa tumor-associated antigen in the sera of melanoma patients. Int J Cancer 45: 1065
11. Fritze D, Kern DH, Drogemuller CR, Pilch YH (1976) Production of antisera with specificity for malignant melanoma and human fetal skin. Cancer Res 36: 458
12. Herlyn M, Rodeck U, Mancianti M, Cardillo FM, Lang A, Ross AH, Jambrosic J, Koprowski H (1987) Expression of melanoma-associated antigens in rapidly dividing human melanocytes in culture. Cancer Res 47: 3057
13. Kopald KH, Gupta RK, Bauer RL, Morton DL (1990) Diurnal variation in the excretion of a tumor-associated antigen into the urine of cancer patients. Proc Am Assoc Cancer Res 31: 261
14. Lando P, Gabriel J, Berzins K, Perlmann P (1980) Determination of the immunoglobulin class of complement-dependent cytotoxic antibodies in serum of D23 hepatoma-bearing rats. Scand J Immunol 11: 253
15. Liao S-K, Kwong PC, Thompson JC, Dent PB (1979) Spectrum of melanoma antigens on cultured human malignant melanoma cells as detected by monkey antibodies. Cancer Res 39: 183
16. McCabe RP, Ferrone S, Pellegrino MA, Kern DH, Holmes EC, Reisfeld RA (1978) Purification and immunologic evaluation of human melanoma-associated antigens. J Natl Cancer Inst 60: 773
17. Metzgar RS, Bergoc PM, Moreno MA, Seigler HF (1973) Brief communication: melanoma-specific antibodies produced in monkeys by immunization with human melanoma cell lines. J Natl Cancer Inst 50: 1065
18. Old LJ (1981) Cancer Immunology: the search for specificity – G. H. A. Clowes Memorial Lecture. Cancer Res 41: 361
19. Nores GA, Dohi T, Taniguchi M, Hakomori S-I (1987) Density-dependent recognition of cell surface GM3 by a certain anti-melanoma antibody, and GM3 lactone as a possible immunogen: requirements for tumor-associated antigen and immunogen. J Immunol 139: 3171
20. Rote NS, Gupta RK, Morton DL (1980) Tumor-associated antigens detected by autologous sera in urine of patients with solid neoplasms. J Surg Res 29: 18
21. Stuhlmiller GM, Seigler HF (1975) Characterization of a chimpanzee anti-human melanoma antiserum. Cancer Res 35: 2132
22. Viza D, Phillips J (1975) Identification of an antigen associated with malignant melanoma. Int J Cancer 16: 312